

Application Serial No. 10/522,706  
Amendment dated 06 July 2007  
Reply to Office Action dated 16 April 2007

*AMENDMENTS TO THE CLAIMS*

This listing of claims will replace all prior versions, and listings, of claims in the application.

***Listing of Claims***

Claim 1 (Currently Amended): ~~Use of at least one proteasome inhibitor for the manufacture of a medicament for the prevention, onset therapy, acute therapy and/or regression of diseases associated with endothelial dysfunction A method of preventing or treating diseases associated with endothelial dysfunction which comprises administering a therapeutically effective amount of at least one proteosome inhibitor to an individual in need thereof, wherein the amount is effective to enhance the expression of endothelial nitric oxide synthase (eNOS) and wherein the amount is in a nanomolar range.~~

Claim 2 (Currently Amended): ~~Use The method according to claim 1, wherein the diseases associated with endothelial dysfunction are non-insulin related diseases.~~

Claim 3 (Currently Amended): ~~Use The method according to claim 1, wherein the endothelial dysfunction is associated with atherosclerosis, in particular coronary sclerosis and coronary artery disease.~~

Claim 4 (Currently Amended): ~~Use The method according to claim 1, wherein the endothelial dysfunction is associated with heart failure.~~

Claim 5 (Currently Amended): ~~Use The method according to claim 1, wherein the endothelial dysfunction is associated with diseases selected from the group comprising ischemic~~

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diseases such as peripheral arterial occlusive disease, e.g. critical leg ischemia, myocardial infarction and ischemic diseases of organs, e.g. of the kidney, spleen, brain, and lung.

Claim 6 (Currently Amended): Use The method according to claim 1, wherein the proteasome inhibitor is selected from a group comprising:

- a) naturally occurring proteasome inhibitors comprising:  
peptide derivatives which have a C-terminal epoxy keton structure,  $\beta$ -lacton-derivatives, aclacinomycin A, lactacystin, clastolactacystein;
- b) synthetic proteasome inhibitors comprising:  
modified peptide aldehydes such as N-carbobenzoxy-L-leucinyl-L-leucinyl-L-leucinal (also referred to as MG132 or zLLL), or the boric acid derivative of MG232, N-carbobenzoxy-Leu-Nva-H (also referred to as MG115), N-acetyl-L-leucinyl-L-leucinyl-L-norleucinal (also referred to as LLnL), N-carbobenzoxy-Ile-Glu(OBut)-Ala-Leu-H (also referred to as PS1) [SEQ ID NO:1];
- c) peptides comprising:  
an  $\alpha,\beta$ -epoxyketone-structure, vinyl-sulfones such as, carbobenzoxy-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon or, 4-hydroxy-5-iodo-3-nitrophenylacetyl-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon (NLVS);
- d) Glyoxal- or boric acid residues such as: pyrazyl-CONH(CHPhe)CONH(CHisobutyl)B(OH)<sub>2</sub> and dipeptidyl-boric-acid derivatives;
- e) Pinacol-esters such as: benzyloxycarbonyl(Cbz)-Leu-leuboro-Leu-pinacol-ester.

Claim 7 (Currently Amended): Use The method according to claim 1, wherein the proteasome inhibitor is selected from a group comprising PS-314 as a peptidyl-boric-acid derivative which is N-pyrazinecarbonyl-L-phenylalanin-L-leucin- boric acid (C<sub>19</sub>H<sub>25</sub>BN<sub>4</sub>O<sub>4</sub>); PS-519 as a  $\beta$ -lacton- and a lactacystin-derivative which is 1R-[1S, 4R, 5S] -1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione (C<sub>12</sub>H<sub>19</sub>NO<sub>4</sub>); PS-273 (morpholin-CONH-(CH-

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naphthyl)-CONH-(CH-isobutyl)-B(OH)<sub>2</sub>) and its enantiomer; PS-293; PS-296 (8-quinolyl-sulfonyl-CONH-(CH-naphthyl)-CONH(-CH-isobutyl)-B(OH)<sub>2</sub>); PS-303 (NH<sub>2</sub>(CH-naphthyl)-CONH-(CH-isobutyl)-B(OH)<sub>2</sub>); PS-321 as (morpholin-CONH-(CH-naphthyl)-CONH-(CH-phenylalanin)-B(OH)<sub>2</sub>); PS-334 (CH<sub>2</sub>NH-(CH-naphthyl-CONH-(CH-Isobutyl)-B(OH)<sub>2</sub>); PS-325 (2-quinol-CONH-(CH-homo-phenylalanin)-CONH-(CH-isobutyl)-B(OH)<sub>2</sub>; PS-352 (phenylalanin-CH<sub>2</sub>-CH<sub>2</sub>-CONH-(CH-isobutyl)-B(OH)<sub>2</sub>); PS-383 (pyridyl-CONH-(CHpF-phenylalanin)-CONH-(CH-isobutyl)-B(OH)<sub>2</sub>); PS-341; and PS-1 Z-Ile-Glu(OtBu)-Ala-Leu-CHO [SEQ ID NO:1]; PS-2 [Benzoyloxycarbonyl]-Leu-Leu-phenylalaninal or Z-LLF-CHO or Z-Leu-Leu-Phe-CHO PS-1; PS-519 as a  $\beta$ -lacton- and a lactacystin-derivative which is 1R-[1S,4R,5S]-1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione (C<sub>12</sub>H<sub>19</sub>NO<sub>4</sub>); epoxomicin (C<sub>28</sub>H<sub>86</sub>N<sub>4</sub>O<sub>7</sub>) and eponemycin (C<sub>20</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>).

Claim 8 (Currently Amended): Use The method according to claim 1, wherein the proteasome inhibitor is selected from a group comprising a peptide aldehyde, a peptide boronate, a peptide vinylsulfone, a peptide epoxyketone, a lactacystin, a peptide  $\alpha$ -ketonaldehyde, an  $\alpha$ -ketoamide, an indanonpeptide, a polyalkylenaldehyde, a polyphenol such as cathechin-3-gallate.

Claim 9 (Currently Amended): Use The method according to claim 1, wherein the proteasome inhibitor is selected from a group comprising Z-Leu-Leu-Leu-al (MG132), Z-Ile-Glu(OtBu)-Ala-Leu-al (PS-1) [SEQ ID NO:1], CEP1612, pyrazylcarbonyl-Phe-Leu-boronate (PS-341), dansyl-Phe-Leu-boronate (DFLB), morpholinonaphthylalanin-Leu-boronate (MG273), NIP-Leu<sub>3</sub>-vinylsulfone (NLVS), Tyr-Leu<sub>3</sub>-VS [SEQ ID NO:2], NIP-Leu-Leu-Asn-VS, Ada-Tyr-Ahx<sub>3</sub>-Leu<sub>3</sub>-VS [SEQ ID NO:3], Ada-Lys(bio)-Ahx<sub>3</sub>-Leu<sub>3</sub>-VS [SEQ ID NO:4], Ac(Me)-Ile-Ile-Thr-Leu-EX (epoxomicin) [SEQ ID NO:5], dihydroeponemycin, lactacystin, clasto-lactacystin-beta-lacton (omuralid), PS-519, Ac-Leu-Leu-Nle-al (ALLN), 3,4-dichloroisocoumarin (DCI), 4-(2-aminoethyl)-bezolsulfonylfluorid (pefablock SC), TMC-95-A, gliotoxin, (-)epigallocatechin-3-gallate (EGCG),

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ritonavir, lovastatin, aclacinomicin A (aclarubicin), cyclosporin, wherein Z represents benzyl oxy carbonyl, all represents aldehyde, VS represents vinylsulfone, NIP represents 3-nitro-4-hydroxy-5-iodophenylacetate, and bio represents biotin.

Claim 10 (Withdrawn): Use according to claim 1, wherein the proteasome inhibitor interferes with gene expression of at least one component of the proteasome complex.

Claim 11 (Withdrawn): Use according to claim 10, wherein the proteasome inhibitor interfering with proteasomal gene expression is selected from a group comprising antisense RNA, double stranded RNA and oligonucleotides hybridising with a DNA sequence encoding at least one component of the proteasome complex.

Claim 12 (Withdrawn): Use according to claim 10, wherein the proteasome inhibitor interfering with proteasomal gene expression is selected from a group comprising a knock out construct.

Claims 13-26 (Canceled).

Claim 27 (New): The method according to claim 1, wherein the nanomolar range is between 1 and 100 nanomolar.

Claim 28 (New): The method according to claim 1, wherein a single administration of the proteosome inhibitor produces a long-term enhancement of the expression of eNOS.

Claim 29 (New): The method according to claim 1, wherein the long-term enhancement is for up to ten days.